

**In the Specification**

Please amend the specification section entitled "Tables and Figures" as follows:

**TABLES AND FIGURES**

Table 1 presents rubrospinal neuronal cell counts obtained from individual control and experimental animals with retrograde Fluorogold labeling from the lumbar cord of an adult rat.

Figure 1A presents [(A)] Photomicrograph of a transverse section of spinal cord of an adult rat at the level of T10 left side hemisection lesion, stained with cresyl violet. All lesions were assessed and always resulted in severing the funiculi through which the rubrospinal tract traverses. The contralateral dorsal (dh) and ventral (vh) horns were always left undamaged; the central canal (cc) is labeled for orientation. [(B)] Figure 1B Assessment of visible Fluorogold diffusion in the control treated and immunologically disrupted hemisected spinal cord. Diffusion of the retrograde tracer was measured at the light microscope level at the time points indicated after injection into the lumbar spinal cord (see methods for details). Immunological demyelination did not significantly affect the diffusion of the tracer.

Figures 2A-2D ~~shows~~ show electron photomicrographs of transverse sections through the dorsolateral funiculus after continuous intraspinal infusion of immunological reagents for 7 days. (Figure 2A) Within one spinal segment (<2mm) of the infusion site, large regions of naked, demyelinated axons were visible. Some axons were observed to be associated with monocyte cells (M, e.g. infiltrating macrophage) and or endogenous microglia, some of which also contained myelin ovoids (arrow) or myelin debris. (Figure 2B) On other grids, monocytes and invading polymorphonucleocytes (PMN) could also be seen in close association with demyelinated axons. Macrophages and/or microglia were identified on the basis of their high density endoplasmic reticulum (arrow-heads), and "finger-like" processes. Some monocytes

have laid down basal lamina components such as collagen (Col), which distinguishes them from astrocytes. Multi-lobed nuclei are characteristic of PMNs and facilitate their identification. (Figure 2C) Example of myelin-disruption. This is often observed 4-8mm (1-2 spinal segments) from the immunological infusion site where the axons were still associated with myelin; however, the myelin lamellae were disrupted (delaminated). Some regions of coherence in the myelin wrapping did persist (arrows). (Figure 2D) Example of the appearance of axons within the dorsolateral funiculus after a control infusion of Guinea-pig complement alone. Some non-specific damage of myelin sheathes occurred, especially within one spinal segment of the infusion site; however, the compact nature of the myelin remained intact. Original magnification x 4000 (Figures A, B, D), x10000 (Figure C).

Figures 3A-3G present demonstrations of regeneration of rubrospinal neurons after left-side thoracic hemisection and subsequent immunological myelin suppression treatment. ~~Panels~~ Figures 3A and 3B are photomicrographs of rubrospinal neurons from the same experimentally-treated animal (14 days infusion of serum complement with anti-GalC); Figure 3A is from the uninjured Red nucleus while Figure 3B is from the injured Red nucleus. ~~Panels~~ Figures 3C and 3D are also from same control-treated animal (14 days infusion of serum complement only): Figure 3C is the uninjured Red nucleus and Figure 3D is the injured Red nucleus. Fluorogold injection within the rostral lumbar cord 28 days after injury resulted in the retrograde labeling of uninjured rubrospinal neurons (Figures 3A and 3C) as well as those rubrospinal neurons that had regenerated from the injured Red nucleus (Figures 3B and 3D). ~~(E) and (F)~~ (Figures 3E and 3F) Axotomized rubrospinal neurons were retrograde labeled at the time of injury with the first label RDA (solid arrow heads) and subsequently 28 days later with the second label FG (open arrow heads). Double-labeled (RDA+FG) cells are indicated by an asterisk and represent those rubrospinal neurons that had regenerated after immunological myelin-suppression treatment. Scale bar = 100µm.

Figure 4 shows a relative quantitative assessment of regeneration of rubrospinal neurons after thoracic injury and immunological treatment. Regeneration was assessed by counting FG-labeled cells in alternating tissue sections: those with both multipolar neuronal morphology and FG labeling were deemed to be positive. Percentage regeneration was calculated by comparison of the retrograde labeled cell counts from the injured Red nucleus with the control uninjured Red nucleus within the same animal. For each animal, the degree of lesion was assessed. Filled bar: myelin suppressed; hatched bar: pooled control treated groups. Data shown  $\pm$  s.d.

Figures 5A-5C demonstrates effects of removal of a single complement protein on immunological demyelination. (Figure 5A) Control uninjured spinal cord. Electron photomicrographs of transverse sections through the dorsolateral funiculus indicating the ultrastructure of adult myelin sheaths. (Figure 5B) 7 day infusion with myelin-specific antibody and human complement sera results in a profound myelin suppression. (Figure 5C) The removal of the C3 component of complement results in a lack of myelin-removal, indicating the fundamental role of this protein in either (i) opsonization, or (ii) the propagation of the cascade to the lytic membrane attack complex (MAC), the final lytic pathway complex. It is believed that it is a fundamental and essential requirement of a myelin specific cell surface binding antibody to activate the classical complement pathway for effective transient demyelination.

Figure 6 shows a relative quantitative assessment of regeneration of lateral vestibulospinal neurons after thoracic injury and delayed immunological treatment. Immunological demyelination treatment was delayed for 1 or 2 months after injury as indicated. Regeneration was assessed by counting FG-labeled cells in alternating tissue sections: those with both multipolar neuronal morphology and FG labeling were deemed to be positive. Percentage regeneration was calculated by comparison of the retrograde labeled cell counts from the injured lateral vestibulospinal nucleus with the control uninjured lateral vestibulospinal nucleus within the same animal. For each animal, the degree of lesion was assessed. Filled bar: myelin suppressed; open bar: pooled control treated groups. Data shown  $\pm$  s.d.

~~Figure 7 presents A) Drawing~~ Figure 7A presents a drawing of a dorsal view of the rat central nervous system, indicating the relative origins and course of the rubrospinal tract (RN) and lateral vestibular tract (LVe). Also illustrated (solid line) is the left-side thoracic hemisection lesion (~ T10, line), the immunological infusion site (~ T11, vertical hatching), and the site of the Fluorogold injection (~L1, diagonal hatching). ~~B) Figure 7B is a~~ Figure 7B is a composite photomicrograph of parasagittal sections through the lower thoracic and rostral lumbar spinal cord (T9- L1, rostral is up). Some Fluorogold diffusion can be clearly emanating from the injection site as an intense white "halo", however, this staining rapidly decreased with distance from the site of injection and none was ever visible rostral to T11, the immunological infusion site (i.e. no diffusion to or above the lesion at T10, thus no evidence for any "false" positive retrograde labeling of brainstem-spinal projections). ~~C) Figure 7C is a~~ Figure 7C is a photomicrograph of a transverse section of spinal cord at the level of T10 left side hemisection lesion, stained with cresyl violet. All lesions were assessed and always resulted in severing the funiculi through which the rubrospinal and lateral vestibulospinal tracts traverse. The contralateral dorsal (dh) and ventral (vh) horns were always left undamaged; the central canal (cc) is labeled for orientation. ~~D and E) Non-specific~~ Figures 7D and 7E show non-specific fluorescence associated with blood cells within the lesion and pump implantation sites indicating the degree of damage associated with the lesion and cannula implantation, respectively. Specific Fluorogold fluorescence labeling was never observed at the level of the cannula implantation or hemisection injury.

~~Figure 8 shows~~ Figures 8A-8E show regeneration of lateral vestibulospinal neurons after left-side thoracic hemisection and subsequent immunological myelin suppression treatment. ~~Panels A and B~~ Figures 8A and 8B are photomicrographs of lateral vestibulospinal neurons from the same experimentally-treated animal (14 days infusion of serum complement with anti-GalC); Figure 8A is of the injured lateral vestibular nucleus and B is from the uninjured lateral vestibular nucleus and. ~~Panels C and D~~ Figures 8C and 8D are also from same control-treated animal (14 days infusion of serum complement only); where Figure 8C is the injured lateral

vestibulospinal nucleus and Figure 8D is the uninjured lateral vestibulospinal nucleus. Fluorogold injection within the rostral lumbar cord 28 days after injury resulted in the retrograde labeling of uninjured lateral vestibulospinal neurons (~~B and D~~) (Figures 8B and 8D) as well as those lateral vestibulospinal neurons that had regenerated from the injured lateral vestibulospinal nucleus (~~A and C~~) (Figures 8A and 8C), please see results for further details. ~~Panel E~~ Figure 8E is a drawing of a transverse section through the midbrain indicating the location of the lateral vestibular nucleus (LVe), SpVe = spinal vestibular nucleus, MVe = medial vestibular nucleus, 4V = 4<sup>th</sup> ventricle, FN = facial nerve tract, 7 = 7<sup>th</sup> cranial (facial) nucleus, PFl = paraflocculus. Scale bar = 100µm

Figure 9 shows relative quantitative assessment of regeneration of rubrospinal and lateral vestibulospinal neurons after thoracic injury and immunological treatment. Regeneration was assessed by counting FG-labeled cells in alternating tissue sections; those with both multipolar neuronal morphology and FG labeling, were deemed to be positive. Percentage regeneration was calculated by comparison of the injured nucleus with the contralateral (uninjured) nucleus within the same animal. For each animal the degree of lesion was assessed. Filled bars, experimental; open bars, pooled control groups.

Figure 10 shows a quantitative assessment of regeneration of descending brainstem-spinal axons after chronic lateral hemisection & delayed immunological treatment. Percentages of retrogradely labeled red nucleus (red) and lateral vestibular (green) neurons vs. Contralateral uninjured, after control (PBS, Ab, GpC) treatment (open bars) or immunological disruption/demyelination (filled bars). Expressed as percentage labeled cells in the injured nucleus vs. Uninjured contralateral.

Please amend the specification sections at the pages and lines indicated as follows:

**At page 29, line 18:**

As seen in ~~Figure 5~~ Figures 5A-5C, the removal of the C3 component of complement results in a lack of myelin-removal. This indicates that this protein has a fundamental role in either (i) opsonization, or (ii) the propagation of the cascade to the lytic membrane attack complex (MAC), the final lytic pathway complex.

**At page 41, line 21-22:**

Ten to 12 week old adult female rats (Sprague-Dawley), approximately 200g in weight, were anaesthetized with Ketamine/Xylazine (60mg/kg, 7.5mg/kg respectively). After a limited laminectomy at T10, a left-side spinal cord hemisection lesion was made with micro-scissors and the extent of the lesion was then confirmed by passing a sharp scalpel through the lesion site (~~Fig. 7~~) (Figs. 7A-7E). Immediately after the lesion, an intraspinal cannula was implanted at T11 (n=22 total) and connected to an Alzet osmotic pump (14 day) to subsequently deliver a continuous intraspinal infusion (@ 0.5  $\mu$ l/hr) of serum complement (GIBCO BRL, #19195-015, 33% v/v) along with a complement-fixing IgG antibody to galactocerebroside (either our own polyclonal antibody or Chemicon Intl. Ltd., #AB142, 25% v/v). Cannulae were held in place by means of dental acrylic applied to the vertebral bone. Muscle layers were then sutured over the dental acrylic, and the superficial tissue and skin closed. The extent of the hemisection lesion was always confirmed histologically at the end of the 5-week treatment and recovery period.

**At page 43, line 5-6:**

Twenty-eight days after the hemisection lesion and consequently 14 days after completion of the intraspinal infusion of the immunological reagents, each adult rat was anaesthetized with Ketamine/Xylazine (60mg/kg, 7.5mg/kg respectively). Fluorogold (FG, 100-150nl total volume, 5% w/v in sterile dH<sub>2</sub>O; Fluorochrome Inc. Englewood, CO, USA) was injected (50-75nl) bilaterally into the middle of the spinal tissue at the L1 level, approximately 1cm caudal to the lesion site (~~Fig. 7~~) (Figs. 7A-7E).

**At page 46. lines 12 & 21:**

In all cases, the Fluorogold label (100-150nl) was injected bilaterally within the rostral lumbar cord (1 cm or 2-3 spinal segments caudal to the hemisection injury site, ~~Fig. 7~~ Figs. 7A-7E). We assessed the time course and degree of rostrocaudal diffusion of Fluorogold within the lumbar and thoracic spinal cord of normally myelinated (control) animals and experimentally treated rats (i.e. under demyelinated and myelin disrupted conditions). Random 25  $\mu$ m sections of experimental and control-treated spinal cords (extending from L2 to T8) were examined under a fluorescent microscope using the highest intensity setting of the 100W mercury lamp. Spinal tissue was examined for the extent of Fluorogold diffusion at varying survival intervals after injection, including: 12hr (n=6), 24hr (n=6), 3d (n=6), 5d (n=6) and 7d (n=22). The maximum rostral diffusion distance observed was 4-6 mm (or 1- 1.5 spinal segments) and occurred within a time span of 24h. The degree of Fluorogold diffusion within the lumbar cord did not change over the subsequent time points examined (~~Fig. 7~~) (Figs. 7A-7E).

**At page 47, line 8,**

The extent of the hemisection lesion was assessed in every animal. In all but one experimental and one control-treated animal, the left thoracic spinal cord was hemisected (~~Fig. 7~~) (Figs. 7A-7E). Most importantly, the regions of the rubrospinal tract (dorsolateral funiculus) and the lateral vestibulospinal tract (ventrolateral funiculus) were severed. The right side white matter tracts were always remained intact and undamaged and usually the gray matter of the uninjured side was also undamaged.

**At page 47, line 17:**

As discussed above, the 2 pairs of brainstem-spinal nuclei examined for evidence of retrograde labeling (after spinal cord hemisection and immunological myelin suppression) were the RN and the LVe. These brainstem-spinal nuclei were chosen for their unilateral projection patterns within the thoracic and lumbar cord, enabling comparisons to be made between the retrograde-labeling within an injured nucleus and the uninjured contralateral homologue. Comparing "blind" counts of the number of labeled neurons within each RN (Figs. 3A-3B), the data indicated that  $31.8\% \pm 4.7\%$  ( $n=8$ , range 10-50%) of the injured magnocellular RN neurons had regenerated a sufficient distance into the caudal lumbar cord to incorporate and retrogradely transport the Fluorogold (Fig. 9). In contrast, control treated animals, receiving either the PBS vehicle alone, GalC antibody alone, or serum complement alone did not exhibit a significant amount of RN labeling;  $1.49\% \pm 0.23\%$ , (Figs. 3C-3D; Fig. 9,  $n=13$ , range 0-3). The labeling of some neurons within the injured right RN nucleus may represent the small number of RN that do not project to the opposite side of the midbrain and descend within the ipsilateral (uninjured) cord (Shieh et al., 1983). No retrograde-labeling of cells was observed within the parvocellular region of the RN.



**At page 47. lines 21-22 & page 48, line 3:**

Retrograde-labeling of regenerating LVe neurons was also observed, but only after experimental demyelination and disruption of spinal cord myelin (~~Fig. 8~~). (Figs. 8A-8E). In 8 experimental animals, the mean percentage of regenerating LVe labeling, in comparison to the uninjured contralateral control nucleus, was  $41.8\% \pm 3.1\%$  ( $n=8$ , range 33-49%). In control-treated animals (see above) the percent LVe labeling was  $2.24\% \pm 0.55\%$  (Figs. 5A-5C,  $n=13$ , range 0-6).

**At page 48, line 5:**

Double retrograde labeling of the injured and myelin-suppressed rubrospinal tract was also qualitatively assessed (Fig. 9E—and-F). Large numbers of RDA-positive (first label) magnocellular RN neurons were observed after direct labeling of the lesion site at the time of hemisection injury to the thoracic spinal cord. After intraspinal myelin-suppression and subsequent injection of Fluorogold caudal to the lesion site (see above for details) a small overlapping population of FG-positive neurons was observed (i.e. some neurons were labeled with both RDA and FG). Cells labeled exclusively by the first or the second tracer were also present in every brainstem analysed.